Effects of Eye-Rubbing on Corneal Biomechanical Properties

THESIS

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By

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Abstract

Keratoconus is a disorder characterized by progressive non-inflammatory corneal degeneration that leads to abnormal shape of the cornea which impairs vision. The disease affects about one per two thousand people worldwide. A potential cause of keratoconus is believed to be abnormal eye-rubbing. Previous clinical studies have shown a significant mechanical trauma to the corneal epithelium after rubbing on the eyes in normal human subjects. However, the mechanism by which eye-rubbing could contribute to the development and progression of keratoconus is not well understood. This project aims to study the effects of eye-rubbing on the mechanical properties of the cornea in a canine eye model. Six pairs of dog eyes were collected from a local animal shelter and the simulated eye-rubbing were induced in one eye per pair while the intraocular pressure (IOP) is monitored. Cornea samples were collected after eye-rubbing and tested using a dynamic mechanical analysis (DMA) and ramp test protocols by a Rheometrics System Analyzer (RSA) to compare mechanical responses between rubbed eyes and control eyes. The complex modulus and dynamic viscosity showed a decreased trend in the samples that experienced eye-rubbing as compared to the control samples. These results suggested that the cornea’s ability to resist dynamic loading may be altered after eye-rubbing. The results of this research provided insight into whether corneal biomechanical properties are altered by eye-rubbing which contributes to keratoconus risk.

Dedication

This document is dedicated to my family.

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Fields of Study

Major Field: Biomedical Engineering

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Chapter 1: Introduction

Keratoconus is indicated by a cornea with a conical shape, significant thinning of the corneal stroma, and breaks in the lamellae of the Bowman's layer, which is a thin layer between corneal stroma and epithelium [1, 4]. A schema graph described the different layers of cornea (Figure 1). The abnormal deformation of the cornea can severely impair visual acuity (Figure 2) [1].

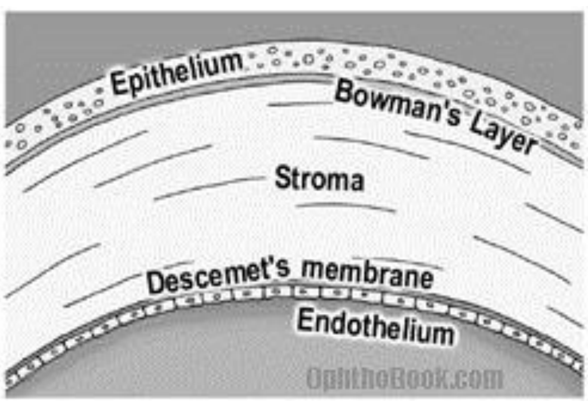

Figure 1.Corneal Layers (www.ophthobook.com)

A chronic habit of abnormal rubbing has been associated with keratoconus [5]. Yeniad *et al* reported that the number of patients with keratoconus who reported rubbing their eyes varied from 12% to 80%, and 48.2% of these patients reported rubbing both eyes vigorously [7]. The principle rubbing-related forces are the compressive loading over a fairly stable palpebral conjunctiva and the shear loading during eye rubbing [4]. Abnormal rubbing caused the upper palpebral conjunctival mast cell degranulation in a rat eye study [3]. Moreover, Keratocyte apoptosis has been shown to be associated with corneal wounding which may be the underlying pathophysiologic mechanism for the development of keratoconus [3].

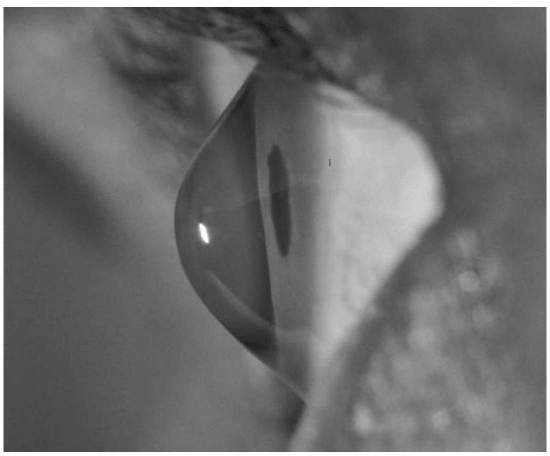

Figure 2. “Conical Cornea” in Keratoconus Patients

Several *in vivo* eye rubbing tests of human subjects have been conducted to study the effect of rubbing on corneal properties. Previous investigations have demonstrated that values of corneal biomechanical measurement are significantly lower in keratoconic eyes than in normal eyes [6]. Liu *et al* reported that corneal hysteresis (reflects viscoelastic or damping properties) and corneal resistance factor (corneal resistance to deformation) were significantly lower after rubbing compared with baseline [8]. The epithelial thickness was recorded to have an 18.4% reduction immediately after rubbing in the study by McMonnies *et al* [4]. The native collagen fiber network is mostly disorganized and lacks the preferred directions and symmetry reported by Meek *et al* (Figure 3) [9].These results indicate certain changes of corneal structures and mechanical properties (viscoelastic properties) after rubbing. In terms of intraocular pressure (IOP), digital pressure on the eye was found to spikes of 50%-130% for an eye with normal IOP (15 mmHg) when the touch was light and of 200%-400% when the touch was firm [10]. Rubbing-related spikes may be much higher because of spiking from both eye closure and compression [2].

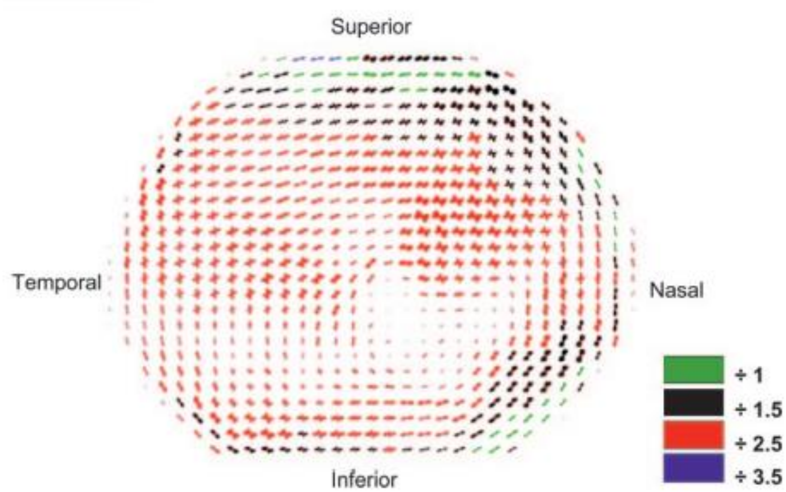

Figure 3. Changes in Collagen in keratoconus cornea

However, the mechanical properties were hard to test *in vivo*. Some parameters, such as corneal hysteresis (CH) can be affected by IOP while rubbing *in vivo*. It is possible that the observed change of CH could be caused by the change in IOP, rather than the changes of the corneal properties. Moreover, corneal hysteresis and resistance factor measured by an Ocular Response Analyzer *in vivo* cannot provide a relative comprehensive description of the viscoelastic properties of corneas.

This project aims to study how corneal mechanical properties (viscoelastic properties) will be changed after eye-rubbing. The application of compression and shear force to the cornea by eye rubbing may results in changes to the viscoelasticity of corneal tissue, thereby changing the ability to resist dynamic loading. Moreover, the loss of epithelium caused by rubbing may aggregate corneal swelling *ex vivo*, thereby leading to a significant thickness increase of rubbed eyes compared to un-rubbed eyes.

Chapter 2: Materials and Methods

2.1 Sample Collection

Six pairs of canine eyes from six dogs were collected within 30 min of euthanasia from a local animal shelter. The globes were transported and stored in sealed tubes filled with phosphate buffered saline (PBS) on ice. All experimentation was conducted within 20h of euthanasia.

Canine eyes were used in this study because their corneal thickness was close to that of human eyes. In addition, canine eyes can be obtained immediately after death and tested within hours to minimize swelling and other postmortem changes, which is important for preserving corneal properties close to the *in vivo* conditions.

2.2 Rubbing Test

Central corneal thickness were measured immediately by a ultrasonic pachymeter (PACHETTE2, DGH Technology,Inc.,Exton,PA) and whole globes were stored in the cornea preservation medium till testing to prevent corneal swelling. Cornea preservation medium contains Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO), 10% Dextran T-40 (MP Biomedicals,LLC,Solon,OH), 1.35% chondroitin sulfate (Sigma, St. Louis, MO), 2% fetal bovine serum (HyClone Laboratories.Inc.,South Logan,UT), 0.1 mM nonessential amino acids (GIBCO,Grand Island, NY), 0.5% penicillin-streptomycin (HyClone Laboratories.Inc.,South Logan,UT) and HEPES buffer (Sigma, St. Louis, MO). The pH is adjusted to 7.0-7.4. A similar receipt was used by Silverman *et al* [19].A higher concentration of dextran was used to achieve a stronger effect to reduce corneal swelling.

Experimental Setup is described in Figure 4. One eye of each pair was randomly chosen as the rubbed eye and the other was control eye. The whole global was put in a custom built holder and fixed by two gage 25 needles.

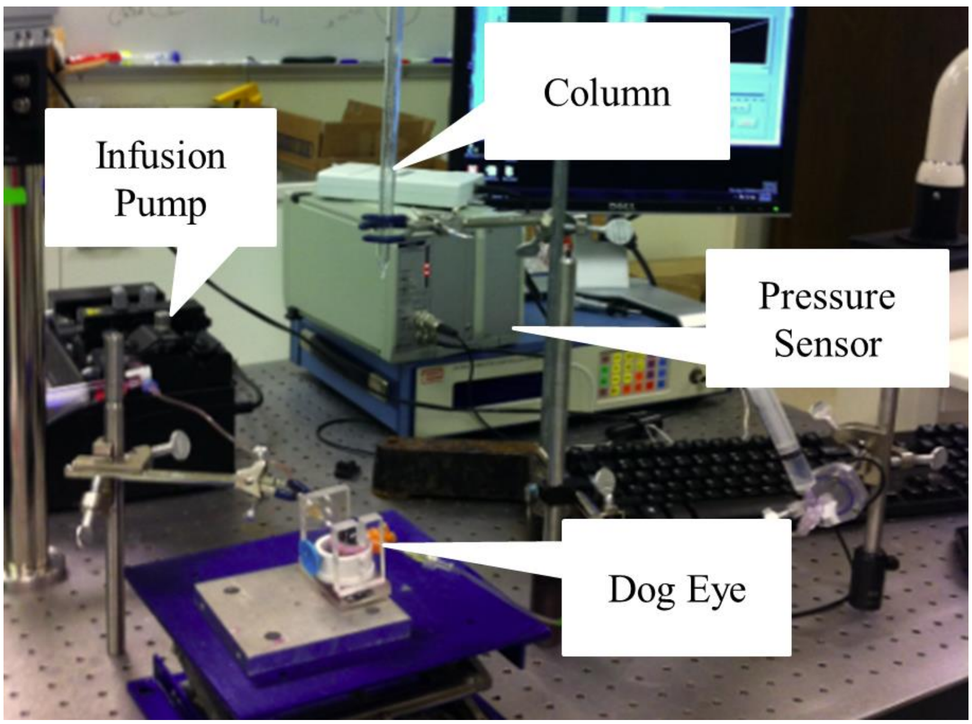

Figure 4. Experimental Setup

Another two gage 20 needles shown in Figure 5 went into the eyeball. Needle A connects the infusion pump or the column to vitreous chamber of the eye and needle B connects to the pressure sensor to the arterial chamber of the eye. The eyes were subjected to preconditioning twice consisting infusion of 30 μL/min for 45 sec and withdraw of 30 μL/min for 45 sec and increased the baseline IOP (15mmHg) to 25 mmHg and adjusted to 15 mmHg afterwards. After precondition, needle A switched to the column filled with corneal preservation medium for the baseline IOP control for rubbed and control eyes. Rubbed eyes or control eyes were randomly done first.

Eye rubbing was conducted by rubbed by an index finger wearing latex glove between nasal and temporal direction of the cornea for 40 sec (A previous study has shown that a 20 sec eye-rubbing can lead to significant changes of the properties of the corneal *in vivo*

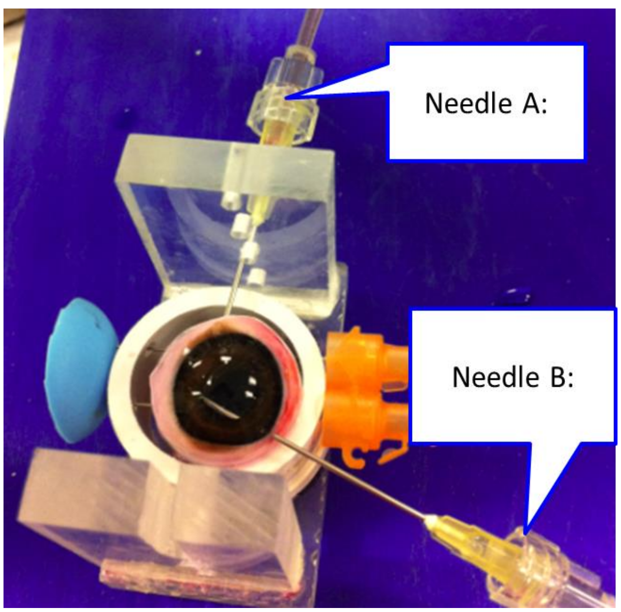

Figure 5 Experimental Whole Global

[8]). The frequency of rubbing is approximately 3 Hz and the magnitudes of forces will be monitored by the spikes of IOP shown in a customized Labview (Labview 2011, NI Corp., Austin, TX) rountine program interface. The spikes of IOP whiling rubbing were 40 mmHg, compared to baseline IOP of 15 mmHg. IOP was and saved for future data analysis.

For control eyes, IOP was adjusted to baseline (15 mmHg) and recorded for 40 sec. Table 1 describes the experimental design of rubbed eyes and control eyes, preservation medium was dropped on the corneas to moisten and reduce corneal swelling during all the recordings of both rubbed eyes and control eyes. After eye rubbing test and IOP measurement, the eyeballs were dissected and corneal buttons were put in the sterilized wells filled with mineral oil to prevent swelling.

<table>
<tr><th colspan="2"></th><th>Targeted IOP (mmHg) during treatment</th><th>Rubbing time (sec)</th></tr>
<tr><td rowspan="2">Group 1
(4 pairs of eyes)</td><td>Control eyes</td><td>Keep at 15</td><td>0 (No rubbing)</td></tr>
<tr><td>Treatment eyes</td><td>spikes are around 30</td><td>40</td></tr>
<tr><td rowspan="2">Group 2
(4 pairs of eyes)</td><td>Control eyes</td><td>Keep at 15</td><td>0 (No rubbing)</td></tr>
<tr><td>Treatment eyes</td><td>spikes are around 60</td><td>40</td></tr>
</table>

Table 1. Experimental Design

2.3 Mechanical Test

Dynamic mechanical analysis (DMA) is one of the standard methods to determine the viscoelastic properties of a material by applying a small amplitude, cyclic strain and observing the cyclic stress response. Measured parameters used in calculating the dynamic properties are shown in Figure 6 [11]. For the parameters, δ is the phase shift between stress and strain, ε_o is strain input amplitude and σ_o is stress output amplitude. The complex modulus represents the overall resistance to deformation under dynamic loading. It is composed of the storage modulus (elastic component) and the loss modulus (viscous component) using the linear theory of viscoelasticity [12]. The complex modulus E* is defined as

$$E{*}=\frac{\sigma}{\varepsilon} = E' + iE'' \qquad (2.1)$$

Where E' is the storage modulus and E'' the loss modulus

Another important viscoelastic parameter, tan(δ) represents the damping ability of the tissue and can be quantified by the ratio of the loss modulus and storage modulus

$$Tan\ (\delta) = \frac{E''}{E'} \qquad (2.2)$$

Dynamic viscosity is described as:

$$\eta' = \frac{E''}{\omega} \qquad (2.3)$$

Where ω is the angular frequency

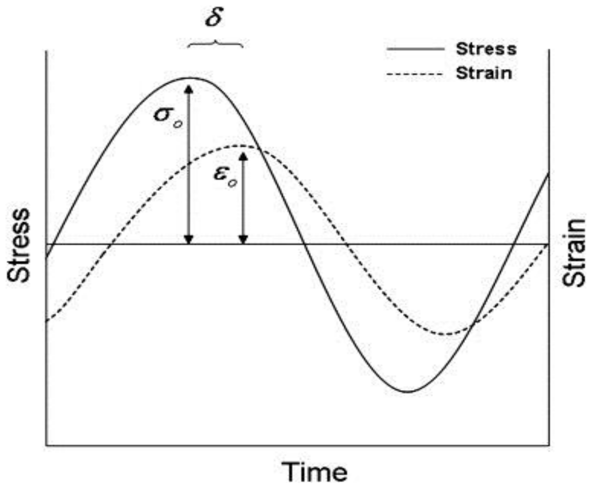

Figure 6. Measured Parameters in DMA of Cornea

DMA testing was performed by a Rheometrics System Analyzer (RSA III, TA Instruments, New Castle, DE) using a published protocol [11]. Thicknesses of the corneas were measured by the pachymeter again before mechanical testing. Cornea strips were excised from the four rubbed and four control corneas by a custom built parallel blade. The widths of strips were measured by a micrometer caliper. DMA was conducted at 2 grams of preload and a strain range of ± 0.15%. The cornea samples were carefully mounted (figure 7) to ensure good alignment and moistened by cornea preservation medium during testing.

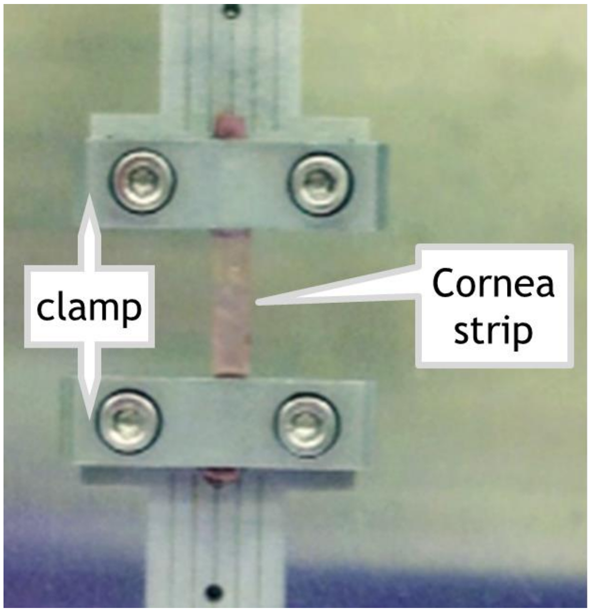

Figure 7. Sample Clamping. A force transducer on the top measures and transfers signals

Ramp tensile test was conducted after DMA and samples were relaxed and applied 0.75 grams of preload. Strain rate was 0.1% per second. The stress/strain data obtained from ramp testing was fit to Fung's standard exponential model [11]

$$\sigma = A \cdot (1 - eB\varepsilon) \tag{2.4}$$

The constants A and B were fitted by a least squares method. The secant modulus at 1.0%, 2.0% and 3.0% strain were calculated as a stiffness measure for comparison among rubbed samples and control samples.

2.4 Statistical Analysis

Data was presented as mean ± standard deviation. Significance was at $p < 0.05$. Statistical analysis was performed using Excel 2010 (Microsoft Corp. Redmond, WA) and Minitab 16 (Minitab Inc. State College, PA). Paired t-test were conducted to compare Complex modulus, dynamic viscosity, tan(δ) and secant modulus at 3% strain between rubbed eyes and control eyes. Thickness of corneas were compared before and after rubbing (or IOP-recording for control eyes) before mechanical testing , and compared between control and rubbed eyes before mechanical testing. The sample size that needs to show a significant difference of complex modulus between rubbed eyes and control eyes was estimated in Minitab 16.

Chapter 3: Results

3.1 Corneal Thickness

The average central corneal thicknesses (CCT) measured by the pachymeter were 590±28 μm for control eyes and 572±47 μm for rubbed eyes before any testing. However, after rubbing test and before mechanical test, average CCT for control eyes were 650±41 μm and for rubbed eyes were 718±48 μm. Significant difference was found (*: P=0.01) between control and rubbed eyes after rubbing, and between rubbed eyes (*: P<0.001) before and after rubbing treatment, and between control eyes (*:P=0.009) before and after the baseline IOP measurement. Figure 8 summarizes the average CCT for all the samples before and after rubbing test.

3.2 Rubbing Test

IOP of eye-rubbing was recorded 32.11±1.39 mmHg for rubbed eyes and was 15.05±0.025 mmHg for control eyes. The spikes of IOP were in the range of ±3.93 to ±6.74 mmHg for all six rubbed eyes. The average central frequency for rubbing was 2.81 Hz, which was analyzed by a fast Fourier transform analysis. The small standard deviation of IOP (1.39 mmHg) while rubbing between different eyes showed a good consistency of eye-rubbing. The targeted IOP spikes while rubbing were 40mmHg and an example of recorded IOP for one rubbed and one control eye (same pair) are shown in Figure 9 and Figure 10. The IOP for the rubbed eye (Dog Ca3, left eye) was 31.53±5.07 mmHg and the central frequency of rubbing was 2.61 Hz. The IOP for the control eye

(Dog Ca3, right eye) was 15.02±0.02 mmHg. Rubbing test was conducted consistently as much as possible for different pairs of eyes.

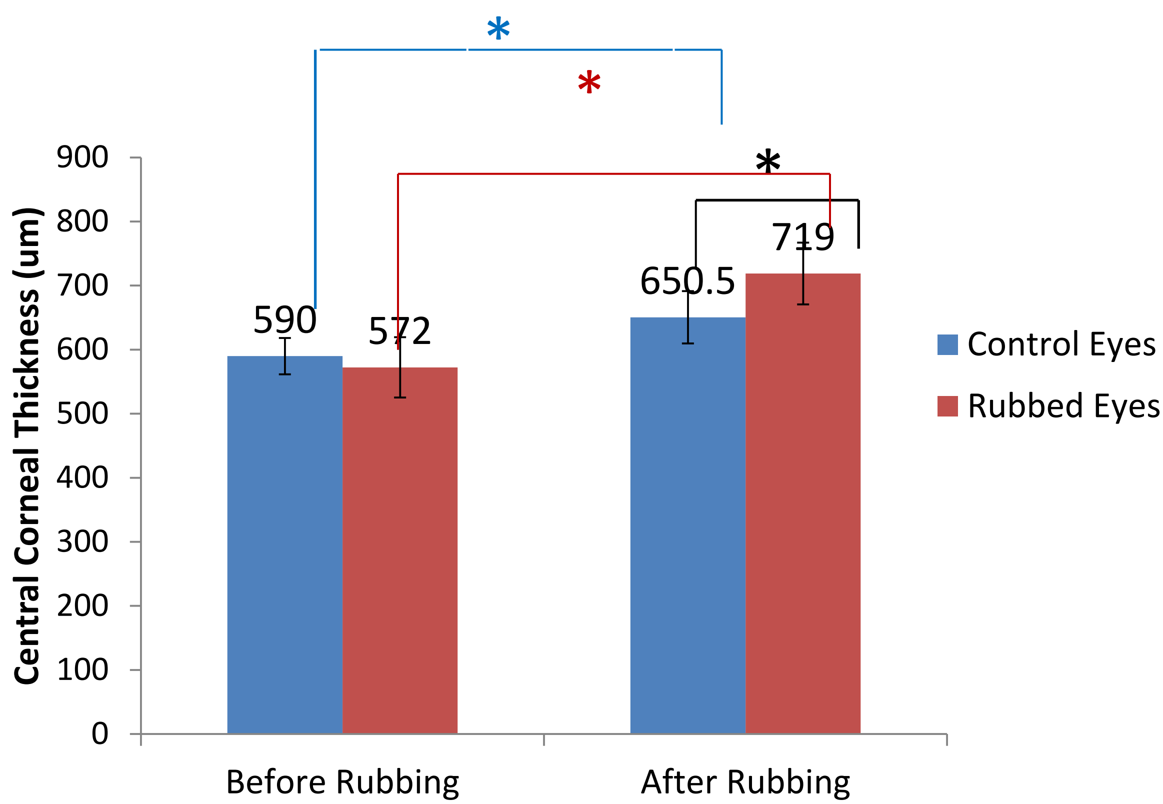

Figure 8. Average CCT for rubbed and control eyes

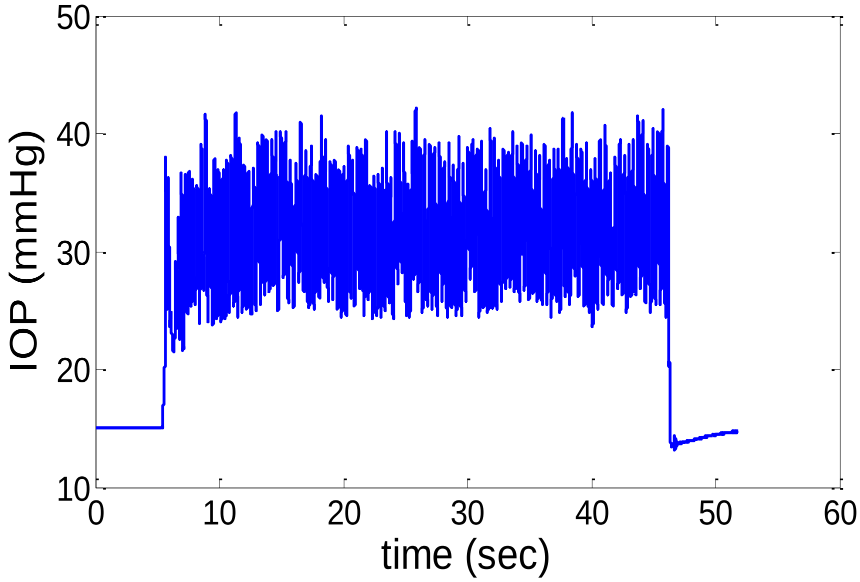

Figure 9. Recorded IOP of Rubbed Eye

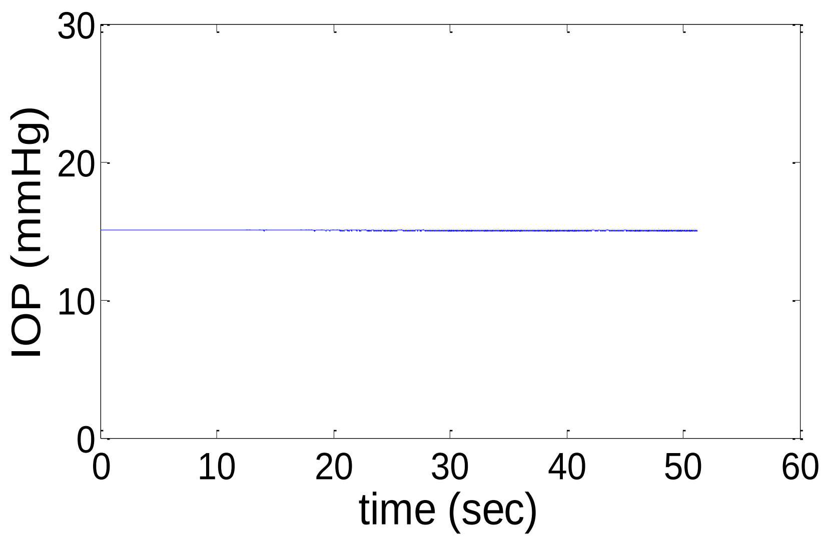

Figure 10. Recorded IOP of Control Eye

3.3 Mechanical Test

The complex modulus from DMA for control eyes was 3.324±0.6 MPa and 2.644±1.1 MPa for rubbed eyes. The dynamic viscosities were 0.093±0.04 MPa·s and 0.071±0.04 MPa·s for control and rubbed eyes, respectively. Moreover, the tan(δ) was 0.160±0.01 for control eyes and 0.163±0.05 for rubbed eyes. A decrease trend of the complex modulus and dynamic viscosity was noticed for rubbed eyes compared to control eyes although no significant difference ($P<0.05$) was found. Figure 11 summarized all three parameters got from DMA for six control eyes and six rubbed eyes.

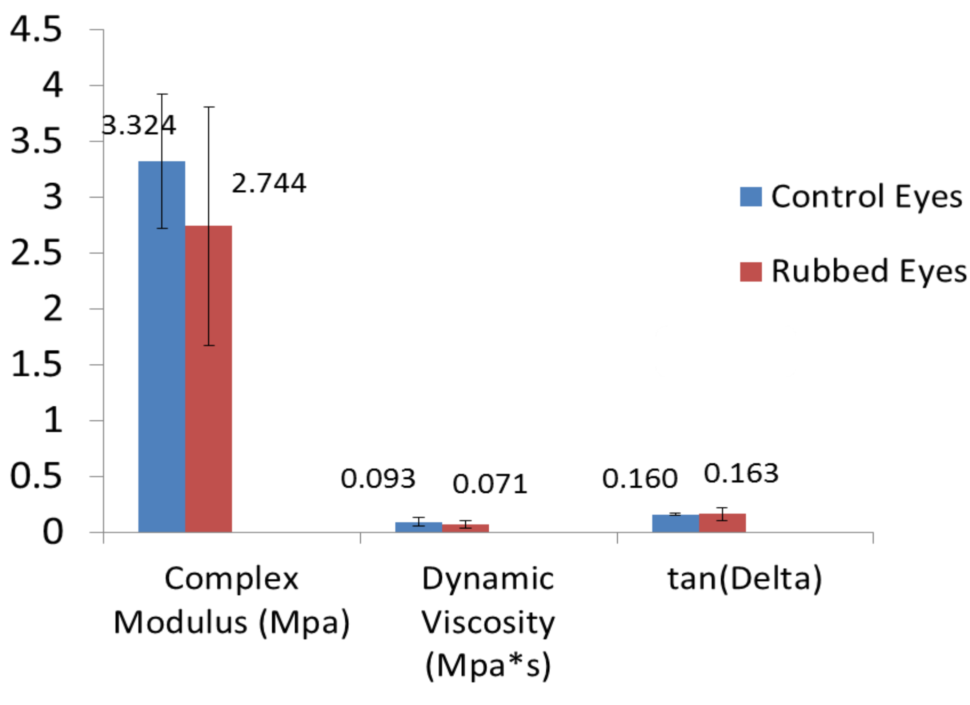

Figure 11. Parameters from DMA Testing (N=6)

Average strain and stress from ramp tensile test were calculated from all 12 corneal samples (six control and six rubbed eyes) and plotted, shown in figure 12. The standard deviations of stress at 1%, 2% and 3% strain were added. The rubbed eyes showed a decrease trend stress at the same level of strain compared to control eyes. The secant modulus at 3% strain were compared and shown in Figure 13. Secant modulus was 3.884±1.98 MPa for control eyes and 3.776±2.53 MPa for rubbed eyes. No significant difference ($P<0.05$) was found between rubbed eyes and control eyes. Table 2 summarized all the parameters from mechanical tests for all six pairs of eyes. P-values were got from paired-t test between control eyes and rubbed eyes for each parameter.

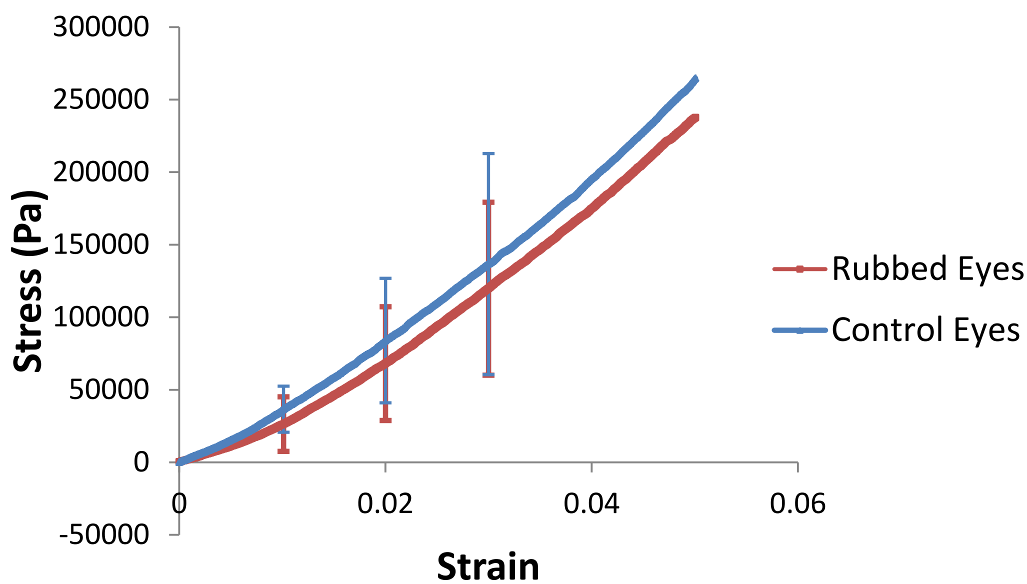

Figure 12. Average Stress vs. Strain for Control Eyes and Rubbed Eyes in Ramp Tensile Test. (N=6)

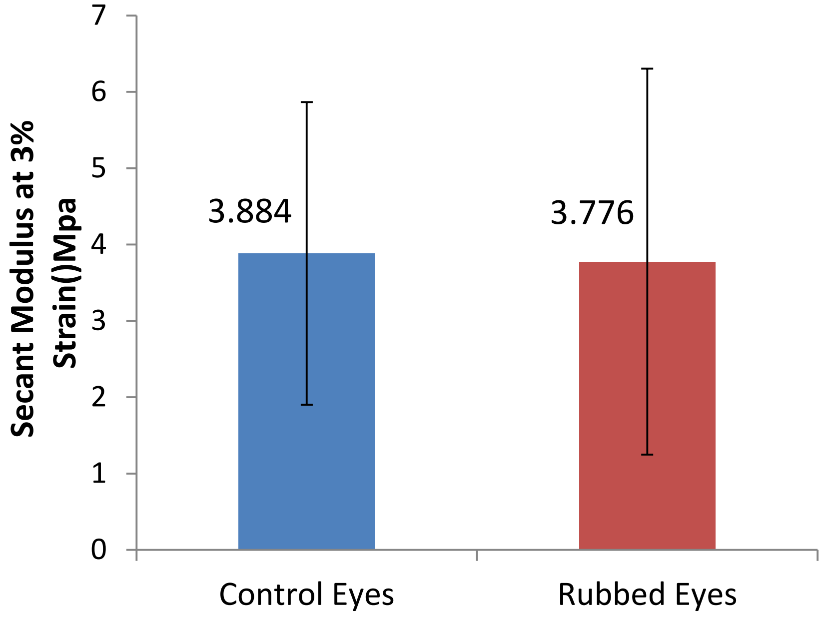

Figure 13.Average Secant Modulus at 3% Strain in Ramp Test. (N=6)

	Complex Modulus (MPa)	Dynamic Viscosity (MPa*s)	tan(Delta)	3% secant modulus (MPa)
Control Eyes (six)	3.324±0.6	0.093±0.04	0.160±0.01	3.884±1.9
Rubbed Eyes (six)	2.644±1.1	0.071±0.04	0.163±0.05	3.776±2.5
P-values	0.233	0.212	0.882	0.900

Table 2. Parameters from mechanical testing (DMA and ramp tensile test) (N=6).

Chapter 4: Discussion

The average Central Corneal Thickness (CCT) of six control eyes was similar to that of six rubbed eyes when tested just after collection. However, average CCT increased about 10% for control eyes (significantly different, P=0.01) and about 25.6% for rubbed eyes (significantly different, P<0.001) after rubbing or control IOP recording before mechanical testing. Significant difference was found (P<0.01) between control eyes and rubbed eyes before mechanical testing. Although the preservation medium and mineral oil were used to minimize the swelling, some levels of swelling were inevitable. Rubbed eyes and control eyes were conducted the similar treatment expect for the rubbing part, the noticed significant difference of average CCT before RSA indicates the effects of rubbing on corneal swelling. McMonnies *et al* [3] reported a statistically significantly mechanical trauma to the epithelium after 15 seconds of light to moderate rubbing, so in this study, the 40 sec long eye rubbing may caused the trauma to the epithelium of the corneas and aggravated cornea swelling on rubbed eyes. Histology study to analyze the structure changes of epithelium can be done in the future to get a better understanding of effect of eye-rubbing on cornea.

The complex modulus and dynamic viscosity both indicated a decrease trend for rubbed eyes compared to control eyes. Specially, complex modulus and dynamic viscosity decreased of 17.4% and 23.9%, respectively for rubbed eyes compared to control eyes. Corneal epithelium and epithelial cells behave viscoelastically [14].When mechanical stress is removed from a viscoelastic material, and there is an instantaneous recovery of the elastic deformation followed by slow recovery of the viscous creep [15]. Plastic deformation in response to rubbing may involve rupture of the cell membrane and loss of cytoplasm [3]. Although no significant difference found in complex modulus and dynamic viscosity, the decrease trend of the two parameters for rubbed eyes indicated the interesting points for future study of the cornea ability to resist dynamic loading and change of viscosity after eye-rubbing. No a significant different ($P<0.05$) found in $\tan(\delta)$ between control eyes and rubbed eyes.

The average strain vs. stress figure (Figure 12) indicated a decrease trend of scant modulus of rubbed eyes compared to control eyes. The secant modulus at 3% for rubbed eyes was slightly lower than that of control eyes. The secant modulus found in this study (control eyes: 3.88±1.98 MPa; rubbed eyes: 3.77±2.53 MPa) were out of the range of past report on canine corneas (1.54±0.74 MPa) [16]. This range deviation may be caused by systematic errors of mechanical testing using RSA, but won't affect the properties comparisons between rubbed eyes and control eyes.

This study has the following limitations. First, the sample size (N=6) was small, which may partially explain why no statistical significant difference ($P<0.05$) for complex modulus and dynamic viscosity that had noticeable difference between control eyes and rubbed eyes. Minitab power and sample size analysis indicated that a size of 28 was needed to display a significant difference ($P<0.05$) for complex modulus between rubbed eyes and control eyes. Next, epithelium and stroma structure, cross-link fibers may be changed during eye-rubbing, but mechanical testing was not conducted after rubbing treatment due to time limitation. Other changes not caused by eye rubbing directly may be occurred in the interval and affect the mechanical testing results. Future work should attempt to conduct the mechanical testing after eye-rubbing immediately. Moreover, the data showed a big standard deviation for the parameters from mechanical testing. This may be caused by the varied properties of samples and systematic errors of testing. More samples and a better consistency for the mechanical testing should be achieved in the future. Last, the rubbing is conducted by finger simply the forces and frequencies were hard to control. Further work should use a more controllable device with known forces and frequencies achieve a better experimental consistency and study the effects of different vigor and frequency during eye rubbing. A longer time of rubbing and a higher IOP spikes while rubbing should be conducted to achieve a more significant effect of eye-rubbing.

Chapter 5: Conclusion

This study provides preliminary experimental evidences of mechanical properties changes of eyes caused by rubbing *ex vivo*. Six pairs of canine eyes were collected and eye-rubbing test was conducted for six rubbed eyes. IOP for control eyes and rubbed eyes were monitored. The small standard deviation of IOP while rubbing between different eyes showed a good consistency of eye-rubbing. Dynamic mechanical analysis and ramp tensile test were conducted for all samples. No significant ($P>0.05$) difference was found for the mechanical parameters for control eyes and rubbed eyes. However, the decrease trend of the two parameters for rubbed eyes indicated the interesting points for future study of the cornea ability to resist dynamic loading and change of viscosity after eye-rubbing. Significant difference was found between control eyes and rubbed eyes after eye rubbing before mechanical testing, which indicated the effects of rubbing on corneas that aggravated the corneal swelling. The results of this research provided insight into whether corneal biomechanical properties are altered by eye-rubbing which contributes to keratoconus risk. A larger sample size, a better consistency for mechanical testing, a study on changes of corneal epithelial structure and a longer and harder rubbing treatment should be conduct in the future.

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